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PRINCIPAL INVESTIGATOR:
Yin-Yuan Mo

CONTRACTING ORGANIZATION:
University of Mississippi Medical Center
Jackson, MS 39216

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Yin-Yuan Mo

E-Mail: ymo@umc.edu

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University of Mississippi Medical Center
2500 State Street, G652
Jackson, MS 39216

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14. ABSTRACT

The major goal of this application is to determine whether newly identified circular RNAs can serve as novel biomarkers for prostate cancer diagnosis and prognosis. There are three specific aims. First, we will determine whether prostate cancer cells display different patterns of circular RNAs from those of normal tissue. Then, we will determine whether differential expression of circular RNAs can also be detected in cell culture models because we can easily manipulate the levels of circular RNAs in cell culture so that we are able to study their functions. Finally, we will determine whether circular RNAs are differentially expressed in blood/serum samples from healthy individuals and prostate cancer patients.

15. SUBJECT TERMS
microRNA, lncRNA, biomarker

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Introduction

Circular RNAs (circRNAs) are a new type of long non-coding RNAs (lncRNAs). Like classic lncRNAs, circRNAs do not code for protein. However, while classic lncRNAs are linear, circRNAs are circular often through back splicing. Moreover, they often have regulatory functions. For example, circRNAs can serve as endogenous microRNA sponges to neutralize the microRNA function. However, it is not clear whether prostate cancer can exploit this mechanism for its own advantage. We would like to determine whether circRNAs are aberrantly present in prostate cancer compared to normal tissue. Identification of such dysregulated circRNAs would lay a foundation for us to explore their role in prostate cancer and to identify novel prostate cancer biomarkers or therapeutic targets. We hypothesize that prostate cancer may exploit this mechanism for its own advantage and thus prostate cancer may display a very different circRNA pattern from normal prostate tissue. Therefore, the major goal of this application is to determine whether newly identified circular RNAs can serve as novel biomarkers for prostate cancer diagnosis and prognosis.

Body

CircRNAs are aberrantly expressed in prostate cancer. As newly discovered molecules, circRNAs are poorly characterized. Little is known whether they are dysregulated in prostate cancer. Thus, our first step was to characterize these new molecules by profiling. Results indicate that a number of circRNAs are either upregulated (Table 1) or downregulated (Table 2) in tumor tissue as compared to normal tissue.

For example, 15 upregulated circRNAs have over 1.5-fold increase in tumor vs normal with p value <0.05. The expression level of hsa_circRNA_104595 was a 2.7-fold higher in tumor than in normal. To better illustrate how the circular form is formed, we provide the sequence for hsa_circRNA_002143, as shown in Fig. 1 as an example. The top part is the actual sequence and the bottom part is when a circle is formed. Two ends at the junction were highlighted by red and blue, respectively.

On the other hand, 18 circRNAs were at least 2-fold decrease in tumor vs normal. For example, hsa_circRNA_002143 was detected about a 3-fold downregulation in tumor as compared to normal (Table 2). We also provide schematic illustration of hsa_circRNA_002143, as shown in Fig. 2.

CircRNAs are derived from various sources. The origin of these circRNAs varies, ranging from intronic, intragenic to exonic. Intronic circRNAs originate from introns; intragenic circRNAs originate from the regions between two separate genes; and exonic circRNAs originate from exons. Furthermore, these exons can be for coding genes or non-coding genes.

CircRNAs can potentially target microRNAs. One of potential functions for circRNAs is the capability to serve as sponges to neutralize the endogenous microRNAs. In this regard, all of these circRNAs had the potential to target more than one microRNA. We listed one for each in Table 1 and Table 2. This suggests that aberrant expression of these

circRNAs may affect the levels of these microRNAs, thus, contributing to prostate tumorigenesis.

Key Research Accomplishments

- We identified 15 upregulated and 18 downregulated circRNAs from prostate cancer cells through profiling.
- All of these circRNAs carry microRNA binding sites, through which they may regulate the level of endogenous microRNAs.
- We will determine whether any of these circRNA impact tumor cell growth in the cell culture models. We will test whether they are differentially expressed in serum samples from normal and prostate cancer patients such that they may serve as biomarkers for prostate cancer.

Reportable Outcomes

Not yet.

Conclusions

Microarray profiling has identified 15 upregulated and 18 downregulated circRNAs from prostate cancer cells. We are currently determining whether ectopic expression of these circRNAs will impact prostate tumor cell growth and invasion. We will also test their potential as prostate cancer biomarkers.

Table 1, Upregulation of circular RNAs in tumors

Name	Tumor/normal	P-value	circRNA_type	Potential miR binding
hsa_circRNA_104595	2.717446	0.003168	exonic	hsa-miR-412-3p
hsa_circRNA_100790	2.0369969	0.015794	exonic	hsa-miR-20b-3p
hsa_circRNA_104927	1.9599525	0.044262	exonic	hsa-miR-500a-3p
hsa_circRNA_102605	1.9344202	0.002482	exonic	hsa-miR-486-3p
hsa_circRNA_000956	1.926276	0.022407	antisense	hsa-miR-765
hsa_circRNA_000554	1.7791893	0.009459	intronic	hsa-miR-153-5p
hsa_circRNA_100438	1.6898966	0.014578	exonic	hsa-miR-383-3p
hsa_circRNA_101175	1.685527	0.033942	exonic	hsa-miR-374a-3p
hsa_circRNA_103975	1.6771255	0.042994	exonic	hsa-miR-493-5p
hsa_circRNA_103950	1.6427088	0.00164	exonic	hsa-miR-143-5p
hsa_circRNA_102889	1.6353354	0.030357	exonic	hsa-miR-9-5p
hsa_circRNA_102545	1.5942883	0.02303	exonic	hsa-miR-573
hsa_circRNA_103417	1.55695	0.011534	exonic	hsa-miR-597-3p
hsa_circRNA_100213	1.5444917	0.041263	exonic	hsa-miR-345-5p
hsa_circRNA_105037	1.5315459	0.000508	exonic	hsa-miR-197-3p

Table 2, Downregulation of circular RNAs in tumors

Name	Tumor/normal	P-value	circRNA_type	Potential miR binding
hsa_circRNA_002143	0.373893479	0.002055	intragenic	hsa-miR-663a
hsa_circRNA_100477	0.373893479	0.004311	exonic	hsa-miR-134-5p
hsa_circRNA_101164	0.373893479	0.003234	exonic	hsa-miR-103a-2-5p
hsa_circRNA_101615	0.373893479	0.004198	exonic	hsa-miR-197-3p
hsa_circRNA_000911	0.446551827	0.01212	intronic	hsa-miR-449c-3p
hsa_circRNA_104084	0.446551827	0.012523	exonic	hsa-miR-506-3p
hsa_circRNA_000780	0.461434466	0.018459	intronic	hsa-miR-651-3p
hsa_circRNA_102701	0.461434466	0.015917	exonic	hsa-miR-369-3p
hsa_circRNA_104121	0.461434466	0.019856	exonic	hsa-miR-203a-3p
hsa_circRNA_104930	0.461434466	0.019407	exonic	hsa-miR-762
hsa_circRNA_104204	0.478932053	0.03059	exonic	hsa-miR-619-5p
hsa_circRNA_101213	0.485389422	0.033127	exonic	hsa-miR-431-3p
hsa_circRNA_104666	0.486375944	0.034278	exonic	hsa-miR-1468-5p
hsa_circRNA_100750	0.496412771	0.037023	exonic	hsa-miR-1301-3p
hsa_circRNA_000881	0.499530875	0.044016	intronic	hsa-miR-557
hsa_circRNA_102445	0.499530875	0.042097	exonic	hsa-miR-644a
hsa_circRNA_103134	0.499530875	0.044068	exonic	hsa-miR-644a
hsa_circRNA_101336	0.501698841	0.04572	exonic	hsa-miR-320b

CCCAGCCCTGGGGAGCCCCTGTGGAAGTGGAGTCCTTCCTGGTCCACCCCGGTGACCTGCT
GCAGCTTCGCTGTCGGCTGCGGGACGATGTGCAGAGCATCAACTGGCTGCGGGACGGGGTG
CAGCTGGCGGAAAGCAACCGCACCCGCATCACAGGGGAGGAGGTGGAGGTGCAGGACTCCG
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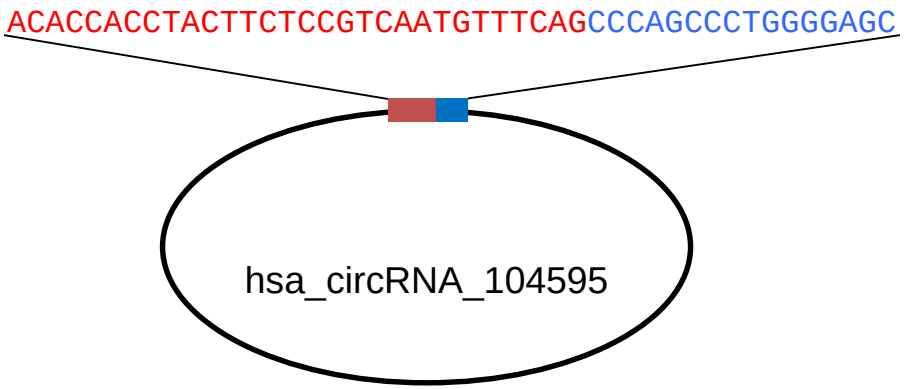

Fig. 1 DNA sequence ofhsa_circRNA_104595. The junction of two ends is highlighted by red and blue, respectively.

AGACAAGGTAGCTCCATAATGGTCAGGCTGGTCTAGAACACCCAACCTGAGGCGTACCACCCAACTTGACCACCCAAAG
TGCTGAGATTAAAGGCGTGAGCTCCGCGTCTGGCCATAACATCTTATCCTATAGAAGCCCAGAGAGGTTAGGCGTCATC
TCACGTGTCGAGGTGATCTCGAACTTTTAGGCTCCAGAGATCCTCCCGCATCGGCCTCCCGGAGTGCTGTGATGACACG
CGTGGGCACGACGGAGTTTCACTCTTGTCGCCCAGGGTGGAGTACGATGGCGGCTCTCGGCTCACCGCACCCTCCGCCT
CCCAGGTTCAAGTGATTCTCCTGCCTCAGCCTTCCCGAGTAGCTGGAATGACAGAGATGAGCCATCGTGCCCGGCTAAT
TTTTCTATTTTTACTACAGATGGGGTTTCTCCATCTTGGTCAGGCTGGTCTTCAACTTCCGACCGTTGGAGAATCTTAA
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GTCGTCGTCCTCCTCCTCCTCCTCCTCCTCCTCCTCCTCCTCCTCCTCCTCCTCCTCTTTCATTTCTTTCAGCTGGGCT
CTCCTACGTGTGTTGCTCTGTTGCTCACGCTGGTCTCAAACTCCTGGCCTTGACGCTTCTCCCGTCACATCCGCCGTCT
GGTTGTTGAAATGAGCATCTCTCGTAAAATGGAAAAGATGAAAGAAATAAACACGAAGACGGAAAGCACGGTGTGAACG
TTTCTCTTGCCGTCTCCCGGGGTGTACCTTGGACCCGGAAACACGGAGGGAGCTTGGCTGAGTGGGTTTTCGGTGCCGA
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CCCCGGCTGGCCTTTGCCGGGCGACCCCAGGGGAACCGCGTTGATGCTGCCTTCGGATCCTCCGGCGAAGACTTCCACC
GGATGCCCCGGGTGGGCCGGTTGGGATCAGACTGGACCACCCCGGACCGTGCTGTTCTTGGGGCACACAGATGAGACGC
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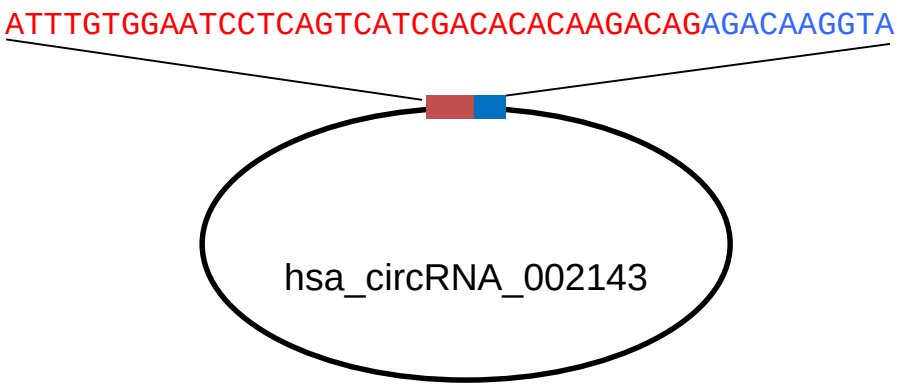

Fig. 2 DNA sequence ofhsa_circRNA_002143. The junction of two ends is highlighted by red and blue, respectively.